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Regulatory effect and mechanism of gastrin and its antagonists on colorectal carcinoma

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Subject headings colorectal neoplasms; gastrin; proglumide; somatostatin; IP_3, Ca^{2+}; protein kinase C; oncogene; AgNORs

Abstract

AIM To explore the effect and mechanism of gastrin and its an tagonists proglumide and somatostatin on colorectal carcinoma and their clinical significance.

METHODS A model of transplanted human colonic carcinoma was established from SW480 cell line in gymnomouse body. The volume and weight of trans planted carcinoma was observed under the effect of pentagatrin (PG), proglumide (PGL) and octapeptide somotostatin (SMS201-995, SMS). The cAMP content of carci noma cell was determined by radioimmunoassay and the DNA, protein content and cell cycle were determined by flow-cytometry. The amount of viable cells was dete rmined by MTT colorimetric analysis, IP_3 content was determined by radioimmuno assay, Ca^{2+} concentration in cell by fluorometry and PKC activity by isotopic enzymolysis. The expression of gastrin, c-myc, c-fos and rasP21 in 48 case s of colorectal carcinoma tissue was detected by the immuno-cytochemistry SP method. Argyrophilia nucleolar organizer regions was determined with argyrophilia stain.

RESULTS The volume, weight, cAMP, DNA and protein content in carcinoma cell, cell amount and proliferation index of S and G_2M phase in PG group were all significantly higher than those of control group. When PG was at the concentration of 25 mg/L, the amount of viable cells, IP_3 content and C a^{2+} concentration in cell and membrane PKC activity in PG group were signi ficantly higher than those in control group; when PGL was at a concentration of 32 mg/L, they dropped to the lowest level in PG (25 mg/L) +PGL group, but without significant difference from the control group. The positive expression rate of gastrin, c-myc, c-fos and rasP21 in carcinoma tissue was 39.6%, 54.2%, 47.9% and 54.2% respectively and significantly higher than that in mucos a 3 cm and 6cm adjacent to carcinoma tissue and normal colorectal mucosa. The pos itive expression rate of gastrin of highly-differentiated adenocarcinoma group was significantly higher than that of poorly-differentiated and mucinous adenoc arcinoma groups. The AgNORs count of carcinoma tissue was significantly higher than that in mucosa 3 cm and 6 cm adjacent to carcinoma tissue and norm al colorectal mucosa; and the positive expression of c-myc and c-fos and the A gNORs count in gastrin-positive group was significantly higher than those in gastrin-negative group.

CONCLUSION Pentagastrin has a promoting effect on the growth of transplanted human colonic carcinoma from SW480 cell line. PGL has no obvious effect on the growth of human colonic carcinoma SW480 cell line, but could inhibi t the growth promoting effect of PG on transplanted carcinoma. Somatostatin can not only inhibit the growth of transplanted human colonic carcinoma from SW480 cell line directly but also depress the growth-promoting effect of gastrin on t he transplanted carcinoma. Some colorectal carcinoma cells can produce and secrete gastrin through autocrine, highly-differentiated adenocarcinoma express the highest level gastrin. Endogenous gastrin can stimulate the cell division and pr oliferation of carcinoma cell and promote the growth of colorectal carcinoma regulating the expression of oncogene c-myc, c-fos. Our study has provided experi mental basis for the adjuvant treatment using gastrin antagonist such as PGL, so matostatin of patients with colorectal carcinoma.

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INTRODUCTION

In recent years, some studies indicated that gastrin could promote the growth of some colorectal carcinomas, but gastrin antagonist such as PGL, somatostatin (SS) could inhibit the growth of those colorectal carcinomas. In order to explore the mechanism of the effect and the clinical significance of gastrin and its antagonists on colorectal carcinoma, we established the model of transplanted colonic carcinoma from SW480 cell line in gymnomouse body, and observed the volume and weight of transplanted carcinoma, content of cAMP, DNA, protein and cell cycle of carcinoma cell under the effect of pentagastrin (PG), proglumide (PGL) and o ctapeptide somatostatin (SMS 201-995, SMS). We also observed the effect of PG and PGL on the amount of viable cells, inositol 1, 4, 5-trisphosphate (IP_3) content and Ca^{2+} concentration in cell and protein kinase C (PKC) activity of colonic carcinoma SW480 cell line cultured *in vitro*, and detected the expression of gastrin, cancer genes c-myc, c-fos and rasP21 in 48 cases of colorect al carcinoma tissue by the immunocytochemical SP method and determined the argyr ophilia nucleolar organizer regions count with argyrophilia stain.

MATERIALS AND METHODS

Materials

Human colonic carcinoma SW480 cell line (Sloan Kittin Memorial Cancer Center, US A), BALB/C gymnomouse (Experimental Animal Center of Third Military Medical Univ ersity), pentagastrin (PG, Shanghai Lizhu Chemical Reagent Co.), octapeptide som atostatin (SMS 201-995, SMS, PHARMA, Swiss); proglumide (PGL, No.11 Pharmacenti cal Factory, Shanghai), RPMI1640 culture solution (GIBCO, USA), 3H-myo-inositol (Institute of Atomic Energy, Chinese Academy of Sciences), T-^{32}P -ATP (Ya Hui Biology Engineer Co.), Fura-2/ AM (Sigma, USA), cAMP radioimmu noassay reagent kit (Department of Nuclear Medicine of Shanghai Second Medical University), multiclonal antibody to gastrin and SP kit (ZYMED Co., USA), monoclo nal antibody for c-myc and rasP21, multiclonal antibody for c-fos (Santa Cruz Co., USA).

Experiment methods

Establishment of transplanted carcinoma model The human colonic carcinoma SW480 cell line was resuccitated conventionally. Cell lines with exub erant vitality were selected to form primary transplanted carcinoma, digested with 0.25% trypsin, centrifugated to wipe off the digestive solution, and adjust ed the cell concentration with RPMI1640 cultural solution to 5×10^6/mL. The living cell amount exceeded 99% by trypan-blue stain. Twelve gymnomice were ran domized into six groups. The cell line went through six passages, each time two gymnomice were inoculated. After 0.2 mL of carcinoma cell solution was inoculated to the back of the neck of gymnomouse, the gymnomice were raised in clean room. When the diameter of the carcinoma reached 1.5 cm to 2.2 cm, the gymnomice were killed by severing the cervical vertebra, and the carcinoma mass was stipped bacteria-freely. After preserving specimen for histological and electron microscopical examination, the mass was smashed, ground into cell suspension with RPIM1640 cultural solution, adjusted the cell concentration to 5×10^6/mL, the next passage was started with 0.2 mL of the cell suspension.

Experimental animal grouping After undergoing passages stably-growing human colonic transplanted carcinoma cells were inoculated into thirty gymnomice. They were randomized into six groups and injected with the experimental drugs subcutaneously the next day, two times per day, for 35 days. The gymnomice were killed 24 hours after the last injection. Control group: 0.4 mL normal saline/mouse. PG group: 4 μg PG/ mouse. PGL group: 10 mg PGL/mouse. SMS group: 6 μg SMS/ mouse. PG+PGL group: 4 μg PG and 10 mg PGL/ mouse. PG+SMS group: 4 μ PG and 6 μg SMS/ mouse.

Volume and weight of transplanted carcinoma The long diameter and short diameter were measured at the same time every sixth days after the inoculation, and the measurement lasted six weeks. The volume was calculated by the formula: $V = 1/2a^2b$. The weight of the mass was then determined after decontamination of the non tumor tissue such as blood and fat tissue.

Flow-cytometry Fifteen g transplanted carcinoma tissue was cut and made into single-cell suspension[1], adjusted to a cell concentration of 1×10^8/L, and fixed with 700 g/L alcohol at 4 ℃. The DNA was stained with propidium iodide to redish flourescence and the protein stained with fluorescein iso thiocyanate to green flourescence. Thirty minutes later, the suspension was analyzed on instrument at room temperature. According to Barlogie cell cycle analysis method, the cells were divided into three parts: G_0/G_1 phase, S phase and G-2M phase. Proliferation index (PI) of the cell was calculated as: $PI=(S+G_2M)/(G_0/G_1+S+G_2M)\times100\%$.

MTT colorimetric analysis[2] Suspend the large intestine carcinoma SW480 cells in logarithmic

growth stage in 100g/L bovine serum culture solution to a concentration of 5×10^8/L. The suspension was inocula ted in to a 96-well plate (100 μL/well) and cultivated for 24 h. After the supernatant was wiped off, 100 μL of 5 g/L bovine serum culture solution was added to each well and the first well as zero control, the second one as cell control, which were added with 100 μL of 5 g/L bovine serum culture solution respectively. From the third one on, 100 μL different drugs with different concentrati on were added to each well as follows and repeated 8 times: PG group: the concentration was 6.25, 12.50, 25.00, 50.00 and 100.00 mg/L, respectively. PG (25 mg/L)+PGL group: the concentration of PGL was 8.00, 16.00, 32.00, 64.00 and 128.00 mg/L. They were cultivated in 37 ℃, 50 g/L CO_2 incubator for 72h, each well added with 10 μL of 5 g/L MTT solution 6 hours before termina ting the culture and 100 μL of 200 g/LSDS solution was added at the termination, kept overnight in the incubator, and the absorptivity (A value) at the 570 nm wavelength was determined on the instrument the next day.

Determination of IP_3 content in carcinoma cell Add 15 mCi/L of [^{3}H]-inosito into the suspension of SW480 cell line, cultivate them in 37 ℃, 50 g/L CO_2 incubator for 18 hours, then add LiCl solution, 2 hours later, add 100μL different drugs with different concentration to each well and cultivate 1 minute as follows: PG group: the concentration was 6.25, 12.50, 25.00, 50.00 and 100.00 mg/L, respectively; PG (25 mg/L)+PGL group: the concentration of PGL is 16.00, 32.00 and 64.00 mg/L. Separate IP_3 from the suspension with anion change separation column according to the method described in reference[3,4], then determine its CPM value with hydroflicker.

Determination of PKC activity Determine the PKC activity in human colonic carcinoma SW480 cell line under the effect of PG and PGL according to the method described in reference[5] with isotopic enzymolysis. The grouping and concentration of PG and PGL were the same with IP_3 determination.

Determination of Ca^{2+} concentration Determine the Ca^{2+} concentration in human colonic carcinoma SW480 cell line under the effect of PG and PGL according to the method described in reference[6] with flu orometry. The grouping and concentration of PG and PGL were the same with IP_3 determination.

Specimen Fourty-eight cases of radical resection of colorectal carcinomas collected from August 1996 to April 1997 in our department were chosen as experimental materials. Ten cases of normal colorectal mucosa served as c ontrol group, including 3 cases of inner prolapse of rectum, 2 of volvulus of sigmoid and 5 of sudden death. All the specimens were confirmed by pathological ex amination. Fresh colorectal carcinoma tissue (3 g) and mucosas 3 cm and 6 cm adjacent to carcinoma were cut respectively, fixed with 40 g/L pa raformaldehyde, dehydrated and embedded with paraffin.

Immunohistochemistry method Immunohistochemistry staining of gastrin, c-myc, c-fos and rasP21 was carried out by SP method, referring to the illustration of reagent kit. The first antibody dilution of gastrin was 1:50 , while the dilution of c-myc, c-fos and rasP21 was all 1:100. Microwav e was used to repair the protein of c-myc, c-fos and rasP21. Normal mucosa of antrum was taken as positive control for gastrin, positively stained tissue of hepatic carcinoma served as positive control for c-myc, c-fos and rasP21. PBS solution replacing the first antibody was taken as negative control. Cells containing brown-yellow pellets were considered as positive cells.

Argyrophilia nucleolar organizer regions count (AgNORs) Argyr ophilia staining was carried out according to Hu PH's modified method[7] and observed under ×100 oil microscope. The nucleus background appeared light yellow while the AgNORs granule appeared palm-black. The amount of AgNORs granule of 50-200 cells and the mean value were calculated.

Statistics All the data was expressed as mean± standard deviation ($\bar{x}\pm s$), and analysed with *t* test or one-way analysis of variance; the differences between the rates of different groups were analysed by χ^2 test.

RESULTS

Model of transplanted human colonic carcinoma in gymnomouse

The inoculation of transplanted carcinoma was 100% successful, no gymnomouse died. The inoculation period was 6-8 days, the speed of growth became stable till the sixth generation. At the end of the 5th week, the long diameter of the mass reached 1.6 cm-2.0 cm. It was elliptical in shape and smooth on surf ace in the early stage; while in the advanced stage, the shape became irregular and the surface became nodal. The histological *H.E.* stain and ultrastructure of the transplanted carcinoma had the same pathological feature of human colonic carcinoma.

Effect of PG, PGL and SMS on the transplanted

human colonic carcinoma in gymnomouse

All the gymnomice bearing the transplanted carcinoma survived at the end of the experiment. The volume, weight, cAMP, DNA and protein content in carcinoma cell, cell amount and proliferation index of S and G_2M phase in PG group were significantly higher than those of control group (P<0.05-001), markedly lower in PGL and PG +PGL group in PG group (P<0.05-0.01), yet there was no st atistical difference between PGL, PG+PGL groups and control group (P>0.05) ; and markedly lower in SMS and SMS+PG group than in PG group and control group (P<0.01). The cell amount of G_0/G_1 phase in PG group was obviously lower than in control group (P<0.01), markedly higher in PGL and PG+PGL gro up than in PG group (P<0.01), without statistical difference between PGL, PG+PGL groups and control group (P> 0.05); markedly higher in SMS and SMS+P G group than in PG group and control group (P< 0.01, Tables 1-3).

Table 1 Effect of PG, PGL and SMS on the volume and weight of transp lanted carcinoma ($\bar{x}$±s, n = 5)

Group	Volume (mm^3)	Weight (g)
Control	1766±36	3.04±0.13
PG	1926±98[a]	3.37±0.21[a]
PGL	1750±53[d]	2.98±0.16[c]
SMS	210±13[bd]	0.90±0.14[bdaa]
PG+PGL	1708±59[d]	2.91±0.23[c]
PG+SMS	224±19[bd]	0.95±0.12[bd]

[a]P<0.05, [b]P<0.01 *vs* control group; [c]P<0.05, [d]P<0.01 *vs* PG group.

Table 2 Effect of PG, PGL and SMS on cAMP, DNA and protein content ($\bar{x}$±s, n = 5)

Group	CAMP(pmol/mL)	DNA(dalton)	Protein(dalton)
Control	2.74±0.14	947±16	364±12
PG	3.18±0.23[b]	1004±17[b]	675±18[b]
PGL	2.72±0.15[d]	940±21[d]	356±9[d]
SMS	1.87±0.14[bd]	684±13[bd]	272±11[bd]
PG+PGL	2.82±0.17[c]	940±25[d]	369±14[d]
PG+SMS	1.86±0.17[bd]	687±21[bd]	274±13[bd]

[b]P<0.01 *vs* control group; [c]P<0.05, [d]P<0.01 *vs* PG group.

Table 3 Effect of PG, PGL and SMS on cell cycle and proliferation index (PI)

Group	G_0/G_1 (%)	S (%)	G_2M (%)	PI (%)
Control	64.92±1.72	18.24±1.20	16.84±2.35	35.08±1.72
PG	59.22±1.18[b]	20.16±1.06[a]	20.62±2.05[a]	40.78±1.81[b]
PGL	67.18±2.23[d]	18.04±1.43[c]	14.78±1.09[d]	32.82±2.27[d]
SMS	80.04±2.29[bd]	14.90±1.46[bd]	5.06±1.61[bd]	19.96±2.39[bd]
PG+PGL	67.76±2.41[d]	18.10±1.40[c]	14.34±0.66[d]	32.24±2.41[d]
PG+SMS	80.26±2.73[bd]	15.22±1.78[bd]	4.54±1.25[bd]	19.22±2.73[bd]

[a]P < 0.05, [b]P < 0.01 *vs* control group; [c]P < 0.05, [d]P < 0.01 *vs* PG group.

Effect of PG on the amount of viable cells (A value), IP_3 content (CPM) and Ca^{2+} concentration in cell and membrane PKC activity of SW480 cell line

When PG was at the concentration of 12.5 mg/L, the amount of viable cells, IP_3 content and Ca^{2+} concentration in the cells of PG group were markedly higher than those in control group (P< 0.05) while it was at the conce ntration of 25 mg/L, they all reached the highest value, but plasma PKC a ctivity decreased, and all had statistical difference from those of control grou p (P<0.05-0.01). When PG concentration exceeded 50mg/L, these items did not continue to increase and plasma PKC activity did not continue to decrease, but they were all statistically different from those of control group (P<0.05-0.01, Table 4).

Effect of PG+PGL on the amount of viable cells (A value), IP_3 content (CPM) and Ca^{2+} concentration in cell and membrane PKC activity of SW480 cell line

When PGL was at the concentration of 8 mg/L, the amount of viable cells (A value) of PG (25 mg/L)+ PGL group was markedly smaller than that of PG group (P<0.01), when at the concentration of 16 mg/L, the amount of viable cells, IP_3 content and Ca^{2+} concentration in cell and membrane PKC activity of PG+PGL group decreased and plasma PKC activity increased, all being statistically different from those of control group (P<0.05-0.01). At the concentration of 32mL/L, they decreased to the lowest value and were markedly lower than those in PG group (P < 0.05-0.01), and did not differ significantly from those of the control group (P>0.05). At the concentration of 64mg/L, these it ems in PG+PGL group did not continue to decrease, but they all had statistical difference from those of PG group (P<0.05 - 0.01, Table 5).

Expression of gastric, c-myc, c-fos and rasP21 and AgNORs count in carcinoma tissue

The positive expression rate of gastrin, c-myc, c-fos and rasP21 in 48 cases o f carcinoma tissue was 39.6%, 54.2%, 479% and 54.2% respectively and signif icantly higher than that in mucosa 3cm and 6cm adjacent to carcinoma tissue and normal colorectal mucosa (P < 0.01). The positive expression rate of gastrin of highly-differentiated adenocarcinoma was significantly higher than that of poorly-differentiated and mucinous adenocarcinoma (P<0.05), there was no statistical difference in the positive expression rate of c-myc, c-fos and rasP21 between groups of different pathological types (P>0.05). The AgNORs count of carcinoma tissue was significantly higher than that in mucosa 3 cm and 6 cm adjacent to carcinoma tissue and normal colorectal mucosa (P < 0.01); and the count of mucosa 3 cm adjacent to carcinoma

tissue was signi ficantly higher than that in mucosa 6cm adjacent to carcinoma tissue and normal colorectal mucosa (P<0.05, Tables 6-7).

Relationship between gastrin and the expression of c-myc, c-fos, rasP21 and AgNORs count in carcinoma tissue

The positive expression rate of c-myc and c-fos and the AgNORs count in gastrin-positive group was significantly higher than those in gastrin-negative group ($P < 0.05 - 0.01$), while the positive expression rate of rasP21 in gastrin -positive group was not different from that in gastrin-negative group (P >0.05, Table 8).

Table 4 Effect of PG on VCC, IP_3, $[Ca^{2+}]$ and PKC activity (pmol/min per mg protein) ($\bar{x}$±s, n = 5)

Group(mg/L)	VCC(*A*)	IP_3(CPM)	$[Ca^{2+}]$I(nM)	Plasma PKC	Membrane PKC
Control	1.554±0.009	8.83±1.33	26.36±2.91	2.39±1.30	1.07±0.28
PG6.25	1.555±0.005	10.16±1.33	30.16±2.16	2.36±0.84	1.09±0.16
12.50	1.563±0.010[a]	11.18±0.75[a]	50.69±2.30[a]	2.30 ±0.45	1.15±0.15
25.00	1.580±0.011[b]	16.17±0.75[b]	101.44±2.49[b]	0.91±0.34[a]	2.65±1.21[a]
50.00	1.579±0.008[b]	14.10±1.60[b]	70.63±4.17[b]	0.92±0.26[a]	2.65±0.60[a]
100.00	1.578±0.010[b]	13.00±2.53[b]	62.59±2.59[b]	0.91±0.14[a]	2.66±0.68[a]

[a]P<0.05, [b]P<0.01 *vs* control group.

Table 5 Effect of PG+PGL on VCC, IP_3,$[Ca^{2+}]$i and PKC acti-vity (pmol/min per mg protein) ($\bar{x}$±s, n = 5)

Group	PG(mg/L)	PGL(mg/L)	VCC(*A*)	IP_3(CPM)	$[Ca^{2+}]$i(nM)	Plasma PKC	Membrane PKC
Control	0.00	0.00	1.554±0.009	8.83±1.33	26.36±2.91	2.39±1.30	1.07±0.28
PG	25.00	0.00	1.580±0.011[b]	16.17±0.75[b]	101.44±2.49[b]	0.91±0.34[a]	2.65±0.21[a]
PG+PGL	25.00	8.00	1.553±0.016[d]				
	25.00	16.00	1.551±0.008[d]	9.17±1.47[d]	32.63±2.86[d]	2.33±0.29[c]	1.05±0.09[c]
	25.00	32.00	1.546±0.011[d]	9.00±1.58[d]	31.79±4.41[d]	2.30±0.61[c]	1.05±0.20[c]
	25.00	64.00	1.549±0.011[d]	9.33±1.97[d]	32.45±2.46[d]	2.32±0.17[c]	1.09±0.12[c]
	25.00	128.00	1.549±0.014[d]				

[a]P<0.05, [b]P<0.01 *vs* control group; [c]P<0.05, [d]P<0.01 *vs* PG group.

Table 6 Expression of gastrin, c-myc, c-fos, rasp21 and AgNORs

Group	*n*	gastrin positive (%)	c-myc positive (%)	c-fos positive (%)	rasp21 positive (%)	AgNORs
Carcinoma tissue	48	19(39.6)[b]	26(54.2)[b]	23(47.9)[b]	26(54.2)[b]	7.10±1.48[b]
3cm mucosa	48	2(4.2)	12(25.0)	9(18.8)	10(20.8)	3.65±1.04[c]
6cm mucosa	48	0(0)	7(14.6)	4(803)	6(12.5)	2.88±0.73
Normal	10	0(0)	1(10.0)	1(10.0)	2(20.0)	2.85±0.60

[b]P<0.01 *vs* 3cm, 6cm and normal mucosa group. [c]P<0.05 *vs* 6cm and normal mucosa group.

Table 7 Expression of gastrin, c-myc, c-fos, rasp21 and AgNORs in different pathological carcinoma tissue

Group	*n*	Gastrin positive (%)	c-myc positive (%)	c-fos positive (%)	rasp21 positive (%)	AgNORs
Highly-differentiated	26	14(53.9)[a]	15(57.7)	13(50.0)	15(57.7)	7.12±1.80
Moderately-differentiated	12	4(33.3)	5(41.7)	7(58.3)	6(50.0)	7.25±0.86
Poorly- and mucinous	10	1(10.0)	6(60.0)	3(30.0)	5(50.0)	7.11±1.20

[a]P<0.05 *vs* poorly-differentiated and mucinous group.

Table 8 Relation between gastrin and c-myc, c-fos, rasp21 and AgNO Rs

Group	*n*	c-myc positive(%)	c-fos positive(%)	rasp21 positive(%)	AgNORs
Gastrin-positive	19	15(78.9)[a]	14(73.7)[b]	11(57.9)	7.84±1.30[b]
Gastrin-negative	29	11(37.9)	9(31.0)	15(51.7)	6.22±1.40

[a]P<0.05, [b]P<0.01 *vs* gastrin-negative group.

DISCUSSION

The main physiological function of gastrin is to stimulate the secretion of gastric acid and nourish the gastrointestinal mucosa. In recent years, it has been found that some gastrointestinal carcinomas could express gastrin gene[8,9] and there existed gastrin receptor on the carcinoma cell membrane, and gastrin could stimulate the growth of gastrin carcinoma. So, more and more researchers focus their interest on the relationship between gastrin and colonic carcinoma.

The transplanted human colonic carcinoma model from SW480 cell line in gymnomouse established by us remained the biological characteristics of colonic carcinoma. After subcutaneous injection of 8μg pentagastrin per day for 35 days, the volume and weight of transplanted carcinoma, the cAMP content in carcinoma cell were significantly higher than those of control group, indicating that gastrin promoted the proliferation of transplanted colonic carcinoma. Ishizuka[10] reported that the combination of gastrin and receptor could increase the content of cAMP in carcinoma cell, then transduct external information into cell through the cAMP or protein kinase C pathway to regulate the cell growth and differentiation. Mauss[11]found that the promoting effect of gastrin on colonic carcinoma cell was selective, margarapeptide gastrin stimulated the growth of HT29, LoVo, CoLo32 cell line, but inhibited the growth of T84, HCT116 cell lines. The difference depended on the quality and quantity of cAMP-dependent protein kinase. The increased expression of Type I cAMP protein kinase could promote the differentiation and growth of carcinoma cells, while the decreased expression of Type IcAMP protein kinase or the increased expression of Type II cAMP protein kinase could inhibit the differentiation and growth of carcinoma cells[12]. Baldwin[13] reported that gastrin could promote the growth of 50 percent of the transplanted colonic carcinoma *in vivo*. We also determined the DNA, protein content and cell cycle by flow-cytometry, and found that DNA and protein content in carcinoma cell and the cell amount and proliferation index of S and G_2M phase of PG group were significantly higher than those of control group, while the cell amount of G_0/G_1 phase of PG group was significantly lower than that of control group. It indicated that the mechanism for the promoting effect of gastrin on the cell division and proliferation of the human colonic carcinoma SW480 cell line might be that gastrin could promote the synthesis of cAMP, DNA and protein in carcinoma cells, the cell growth from G_0/G_1 phase to S and G_2M phase, and regulate the cell cycle of colonic carcinoma cells after receptor.

Venter[14] firstly found that there existed high appetency gastrin receptor on the membrane of human colonic carcinoma cell line LoVo and MC-26, both the nutritious effect of gastrin on the mucosa of normal large intestine and the growth-promoting effect of gastrin on large intestine carcinoma were realized through the combination of gastrin and its receptor on the membrane. We found by MTT colorimetric analysis that pentagastrin could promote the increase of viable cell count of colonic carcinoma SW480 cell line and the effect had a dose-effect dependent relationship with the concentration to some extent, along with the in crease of dosage of pentagastrin, its growth-promoting effect did not continue to increase, but inclined to a stable level. This f inding was consistent with the receptor theory, i.e., the receptor had saturation and indirectly proved that the growth promoting effect of gastrin on large intestine carcinoma was intermediated by gastrin receptor.

Some researches indicate that gastrin is a kind of autocrine growth-promoting factor. Hoosein *et al*[15] found that after the colonic carcinoma HCT1 16 and CBS cell line were cultured for 72 hours, the concentration of gastrin was 10.15pg/10^6 cell. Finley *et al*[16] found by the immunocytochemi cal method that at least 50% of the colorectal carcinoma cells expressed gastrin. In our experiment, 19 of 48 cases of colorectal carcinoma expressed gastrin, with a positive rate of 39.6%, which was significantly higher than that in mucosa adjacent to carcinoma and normal mucosa, the expression rate of gastrin in highly-differentiated group was the highest (53.9%), all these indicated that so me colorectal carcinomas could produce gastrin in an autocrine manner. Translational processing of gastrin mRNA to precursor forms of gastrin needs the participation of multiple enzymes and cofactors to produce mature gastrin finally. Therefore, the sound processing enzymes such as peptidylglycine α-amidating monooxygenase and cofactors in well differentiated carcinoma cells may contrib ute to the production of mature gastrin, while the lack of other cofactors or en zymes in poorly-differentiated carcinoma cells may contribute to incomplete processing of precursor forms of gastrin and the difficiency of mature gastrin[17]. So, the gastrin expression of highly-differentiated adenocarcinoma was obviously higher than that of poorly-differentiated one.

We firstly applied the AgNORs technique to the clinical study of the growth-promoting effect of gastrin on large intestine carcinoma. The AgNORs count could mirror the structure and function of nucleolus, the transcription activity of rRNA and the cell proliferation. The AgNORs count of

colorectal carcinoma in our study was significantly higher than that of mucosa adjacent to carcinoma and normal mucosa, while the count of mucosa 3cm adjacent to carcinoma was higher than that of mucosa 6cm adjacent to carcinoma and normal mucosa. It indicated that the DNA in carcinoma cell and mucosa 3cm adjacent to carcinoma was in a disorder state, the regulation of cell proliferation was uncontrolable. We also found that the AgNORs count of gastrin-positive group was significantly higher than that in gastrin-negative group, indicating that endogenous gastrin had growth-promoting effect on some kinds of colorectal carcinomas.

Oncogene c-myc and c-fos is a kind of effect protein of the karyomitosis signal, which can trigger and regulate the transcription of the genes related with proliferation, besides, c-fos can also regulate its own gene expression with a positive feedback and promote the mitosis and proliferation of the cells[18]. Oncogene ras, a kind of GPT protein located at cell plasma and membrane, participates in the signal transduction regulation of various growth factor recept ors. Once being mutant, ras oncogene will be continously activated and obviously promote the mitosis of the cells[19]. Our results revealed that the positive expression rate of gastrin, c-myc, c-fos and rasP21 in carcinoma tissue was significantly higher than that in mucosa adjacent to carcinoma tissue and normal colorectal mucosa, the positive expression rate of c-myc and c-fos in gastrin-positive group was significantly higher than that in gastrin-negative group. It indicated that the growth-promoting effect of gastrin on colorectal carcinoma may be correlated with the activation of oncogenes. Wang *et al*[20] found that administration of gastrin resulted in the rapid appearance of c-myc mRNA in IEC-6 cells, the maximum increase in c-myc mRNA levels was 7.5-fold that of the normal value. Andrea *et al*[21,22]reported that gastrin had a promoting effect on the growth of AR4-2J cell line, and could induce the increase of c-fos mRNA content; after having combined with its receptor, gastrin trige red a series of phophorylation in PKC signal pathway and induced the activication of extracellular signal regulatory kinase ERK 2, the kinase increased the tra nscription activity of EIK-1, then enlarged the expression of c-fos gene and stimulated the cell proliferation. Seva *et al*[23] reported that gastrin stimulated MAP kinase activation in a dose- and time dependent manner, rasP21 may link the MAP kinase pathway to gastrin receptors to trigger the activation.

Phosphatidylinositol signal pathway played an important role in the biological transmembrane transduction, which was intimately correlated with cell proliferation and tumorgenesis[24]. Phosphatidylinositol-4, 5-bisphosphate (PIP-2) was the direct precursor of second messengers IP_3 and PG, and it functioned through IP_3-Ca^{2+} pathway and DG-PKC pathway. The ascendance of dissociate Ca^{2+} concentration was an important mitosis promoting signal and Ca^{2+} correlated with the genesis and growth of many kinds of carcinomas. Protein kinase C was a kind of important kinase in the phosphatidylinositol signal path way, it existed in plasma of static cells by a non activation form, and once it was activated, it moved to cell membrane. The changes of activity were intimately correlated with cell proliferation. In our study, after we applied pentagastrin to colonic carcinoma SW480 cell line, the VCC, intracellular IP_3 content and Ca^{2+} concentration and membrane PKC activity all ascended and were dose-d ependant obviously. But when pentagastrin and antagonist of gastrin were taken together, they remained changed, demonstrating that the growth-promoting effect of pentagastrin on human colonic carcinoma SW480 cell line was correlated with phosphatidylinositol signal pathway and it was through its receptor intermediate function in phosphatidylinositol signal pathway that proglumide antagonized the growth-promoting effect of gastrin on carcinoma cell. Ishizuka *et al*[10] suggested that the combination of gastrin and its receptor activated the membrane phospholipase and then hydrolyzed phosphatide to PI and DG, the latter then activated PKC, and finally Ca^{2+} was released and functioned.

All the findings above and our results indicated that some carcinoma cells produced gastrin by autocrine, after being intermediated by the second messenger in the cells, the expression of oncogene c-myc and c-fos was enlarged, thus promoting the proliferation of the carcinoma cells.

To block any link in the function procedure of gastrin could weaken or inhibit the growth-promoting effect of gastrin on large intestine carcinoma. There were three kinds of gastrin receptor antagonists: proglumide, somatostatin and prostaglandin (PG). Most of the interests were focused on proglumide, amide, the functional group of proglumide could particularly compete gastrin receptor with gastrin. In our research, 10mg proglumide was injected subcutaneously into transplanted carcinoma in gymnomice, 35 days later, compared with control group, there was no statistical difference in the volume, weight, intracellular cAM P, DNA, protein content and cell cycle of transplanted carcinoma in the PG group, but all the value above including cell amount of S and G 2M phase, proliferat ion index of PG and proglumide group were significantly lower than those of PG group, while the cell amount of G_0/G_1 phase was higher than that of PG group. All these

showed that proglumide had no effect on the growth of human colonic carcinoma SW480 cell line but could inhibit the growth-promoting effect of gastrin on transplanted carcinoma. Proglumide has been applied to the clinical practice. K ameyama *et al*[25] reported that seven large intestine carcinoma patients with hepatic metastasis were treated with proglumide (three times daily, each time 400mg) and 5'DFUR (800mg) for 2 years after the resection of the hepatic metastatic mass, at the same time, patients received chemotherapy by hepatic artery encheiresis and followed up for an average of 39 months. The relapse rate of the proglumide+ chemotherapy group was 14% (1/7), while the rate of the chemot herapy alone group was 52% (14/ 26), so they drew a conclusion that the hormono therapy could effectively prevent the relapse after the resection of the hepatic metastatic mass. We used proglumide as an adjuvant drug to treat 25 colorectal patients having radical resection, besides administration of 400mg proglumide three times a day, MFA chemotherapy program was undertaken at the same time, while 25 patients as control group undertook MFA chemotherapy program alone. Follow-up results indicated that the 3-year survival rate of proglumide group (80%) was higher than that in control group (64%), the relapse or metastasis rate of proglumide group (12%) was lower than that in control group (20%), but without statistical differences ($P>0.05$). The 3-year survival rate of Duke's C and D patients in proglumide group (73.3%) was obviously higher than that in control group (42.8%) ($P<0.05$). It indicated that administration of proglumide as adjuv ant therapy for patients with colorectal carcinomas, particularly the middle and late stage ones, could decrease the relapse or metastasis rate and prolong the survival period. But being a kind of weak gastrin receptor antagonist, only when the gastrin receptor level was high, could proglumide have an inhibitory effect. Therefore, its curable effect on large intestinal carcinoma awaits further evaluation more clinical observations.

Somatostatin is a kind of annular peptide hormone secreting by D cell. The main effect of somatostatin is to inhibit the growth, secretion and absorption of the mucosa of the gastrointestinal tract and to inhibit the release of gastrin, secretin, glucagon and growth hormone. Itzeu *et al*[26] found with immunohistochemistry that there existed D cell in the mucosa of colorectal carcinoma, and suggested that D cell in mucosa of large intestine might have local regulatory effect on the secretion of other hormones. Dy *et al*[27] reported that SMS 20.995 could significantly and concentration-dependently inhibit the growth of transplanted human colonic carcinoma from LIM2405 and LIM2412 cell line. We found that the volume, weight, DNA and protein content in carcinoma cell, cell amount and proliferation index of S and G_2M phase in SMS group and SMS+PG group were markedly lower than those in PG group and control group, and markedly higher in PG group than those in control group. The cell amount of G_0/G_1 phase in SMS group and SMS+PG group was significantly higher than that in PG group and control group. This demonstrated that octapeptide somatostatin had a negative regulatory effect on the transplanted carcinoma, and it could not only inhibit the growth of transplanted human colonic carcinoma from SW480 cell line directly but also inhibit the growth-promoting effect of gastrin on the transplanted carcinoma. The half-life period of somatostatin is shorter than two minutes, so it can not be used pharmaceutically, but its analog manually synthesized such as SMS 201-995 and RC-160 have been used in clinical practice. Having treated 55 pati ents with advanced digestive tract carcinomas who could not endure chemotherapy with SMS201-995, Cascinu *et al*[28]found that SMS201-995 could relieve symptom and improve the quality of life and prolong the survival.

Colorectal carcinoma is a common kind of malignant carcinoma. The carcinoma was mostly in middle or advanced stage when patients first came to see a doctor. Treatment of gastrin-sensitive patients with colonic carcinoma with gastrin recept or antagonists such as proglumide and somatostatin as adjuvant therapy is expected to prolong the survival of the patients and to raise the curative effect and to create new approaches for non-cytotoxic therapy such as hormonotherapy of patients with colorectal carcinoma.

REFERENCES

1 Zuo LF. Techniques of the preparation of the flow-cytometry sample. Ed 1.Beijing: *Hua Xia Press*,1989:51-53
2 Wu JG. Practical Clinical immunology test. Edition 1. *Nanjing: Jiangsu Kexue Jishu Press*, 1989:39-41
3 Olivier M, Baimbridge KG, Reiner NE. Stimulus-response coupling in monocytes infected with leishmania.*J Immunol*,1992; 148:1188-1196
4 Huang JQ, Gong FL, Xiong P. The effect of heparin, trifluoperazine on the regulatory effect of noradrenalin on the expression of Iaantigen in rat abdominal giant phagocyte. *Zhongguo Mianyixue Zazhi*, 1994; 10:35-36
5 Yassin RR, Murthy SNS. Possible involvement of protein kinase C in mediating gastrin induced response in rat colonic epithelium. *Peptides*,1991;12:925-927
6 Ishizuka J, Martinez J, Townsena CM. The effect of gastrin on growth of human stomach cancer cells. *Am Surg*,1992;215:528-535
7 Hu PH, Hu DY, Zhuang YH, Zhao ML, Wang RN. The implication of AgNORs in pathological diagnosis of stomach mucosa. *Shanghai Sec Med Uni Transaction*,1989;9:318-320
8 Mongas G, Biagini P, Cantaloube JF. Detection of gastrin mRNA in fresh human colonic carcinomas by reverse transcription polymerase chain reaction. *J Mol Endocinol*,1993;11:223-229
9 Xu Z, Dai B, Dhrura B. Gastrin gene expression in human colon cancer cells measured by a simple competitive PCR method. *Life Sci*,1994;54:671-678
10 Ishizuka J, Townsend JR, Richard J. Effects of gastrin on 3', 5'-

cyclie adenosine monophosphate, inyracellular calcium and phosphatidylinositol hydrolysis in human colon cancer cells.*Cancer Res*,1994;54:2129-2235
11 Mauss S, Niederau C, Hengels K. Effects of gastrin, proglumide, loxiglumide and L-365, 260 on growth of human colon carcinoma cells. *Anticancer Res*, 1994;14(1A):215-220
12 Chochung YS, Clair T, Tortora G, Yokozaki H, Pepe S. Suppression of malignancy targeting the intracellular signal transducing proteins of cAMP: the use of site-selective cAMP analogs antisense strategy and gene transfer. *Life Sci*,1991;48:1123-1132
13 Baldwin GS, Whitehead RH. Gut hormone growth and malignancy. *Baillieres Clin Endocrinol Metab*, 1994;8:185-214
14 Rae-Venter. Gastrin receptor in cultured human cell derived from carcinoma of colon.*Endocrinology*,1981;108:153
15 Hoosein NM, Kiener PA, Curry RC. Evidence for autocrine growth stimulation of cultured colon tumor cells by a gastrin/cholecystokinin-like peptide. *Exp Cell Res*,1990;189:1165-1169
16 Finley GG, Koski RA, Melhem MF, Pipas JM, Meisler AI. Expression of the gastrin gene in the normal human colon and colorectal adenocarcinoma. *Cancer Res*,1993;53:2919-2926
17 Singh P, Xu Z, Dai B, Rajaraman S, Rubin N, Dhruva B. Incomplete processing of progastrin expressed by human colon cancer cells: role of noncarboxyamidate gastrin.*Am J Physiol*,1994;266:G459-468
18 Williams GT, Smith CA. Molecular regulation of apoptosis: genetic controls on cell death.*Cell*,1993;74:777-779
19 Khosravi-Far R, Der CJ. The ras signal transduction pathway. *Cancer Metastasis Rev*,1994;13:67-89
20 Wang JY, Wang HL, Johnson LR. Gastrin stimulates expression of protooncogene c-myc through a process involving ployamines in IEC-6 cells. *Am J Physiol*,1995;269(cell physiol. 38):c1474-1481
21 Andrea T, Yoshiaki T, Catherine S, Dickinson CJ, Tadutaka Y. Gastrin and Glycine extended progastrin processing intermediates induce different programs of early gene activation.*J Biol Chem*, 1995;270:28337-28341
22 Todisco A, Takeuchi Y, Urumov A, Yamada J, Stepan VM, Yamada T. Molecular mechanisms for the growth factor action of gastrin. *Am J Physiol*, 1997;273(Gastrointest Liver Physiol, 36):G891-898
23 Seva C, Chauvel AK, Blanchet JS, Vaysse N, Pradayrol L. Gastrin induces tyrosine phosphorylation of shc proteins and their association with the Grb2/Sos complex.*FEBS Letters*,1996;378:74-78
24 Hill CS, Yreisman R. Transcriptional regulation by extracellular signals: mechanisms and specificity.*Cell*, 1995;80:199-211
25 Kameyama M, Nakamori S, Imaoka S, Yasuda T, Nakano H, Ohigashi H. Adjuvant chemoendocrine chemotherapy with gastrin antagonist after resection of liver metastasis in colorectal cancer.*Gan To Kagakn Ryoho*,1994;21:2169-2171
26 Itzeu BD, Lolova I, Davidoff M. Immunocytochemical and electromicroscopical data on the differentiation of somatostatin-containing cells in human large intestine.*Anat Anz Jena*,1988; 166:77
27 Dy DY, Whitehead RH, Morris DL. SMS201.995 inhibits in vitro and in vivo growth of human colon cancer.*Cancer Res*,1992;52: 917-923
28 Cascinu S, Ferro ED, Catalano G. A randomised trial of octreotide *vs* best supportive care only in advanced gastrointestinal cancer patients refractoryto chemotherapy.*Br J Cancer*,1995;71:97-101

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